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- (54) PARENTERAL MEDICINAL COMPOSITION CONTAINING HUMANIZED MONOCLONAL ANTIBODY FRAGMENT AND METHOD FOR STABILIZING THE SAME
- (57) This invention relates to a parenteral pharmaceutical composition which comprises a humanized monoclonal snilbody fragment, a nononic surface active agent and saccharides, wherein its phi is weakly acidic. The invention also relates to a method for the stabilization of a humanized antibody fragment, which comprises formulating a nonionic surface active agent and saccharities and adulation the off to a weakly scidic

level.

According to the invention, a stable parenteral pharmaceutical composition or paranteral pharmaceutical preparation can be provided, which comprises a humanized monoclonal artifloody fragment and tase no using initiations such as cold place preservation avoiding freezing, transfer and handling avoiding staking, particle removing operation by a fifter when used and the

Description

Technical Field

9 [9001] This invention relates to a stable parenteral pharmaceutical composition which comprises a humanized monoclonal antibody fragment. Particularly, the invention relates to a stable parenteral pharmaceutical composition which comprises Reb fragment of a humanized monoclonal antibody for a fibringogen receptor of a human platelet membrane glycoprotein GPII-5/Illia. The invention also relates to a method for the stabilization of a humanized monoclonal antibody fragment, which comprises formulating a nononic surface active agent and saccharides and adjusting the prit to a weakly acodic layer.

Background Art

[0002] Since the proposal of a method for the mass production of monoclonal antibodies by means of genetic engineering, monoclonal antibodies have been broadly used in the field of medicaments.

10033 Recently, Centoor in the United States has developed "ReoPro (trade name)" comprised of a human-mouse chimaric monoclonal antibody fragment and is providing its as a platelet aggregation inhibitor in the clinical field. The pharmaceutical preparation is a liquid preparation of pit 7.2 containing 2 mg/mil of a chimaric monoclonal antibody fragment, 0.01 M of sodium phosphate, 0.15 M of sodium chiotide and 0.001% of polyscribate 9.0. Since this preparation is a liquid preparation, it is not necessary to use it by dissolving in desilied water for injection or the like prior to use as in the case of freeze-dried preparations. However, this preparation has some limitations in handling it, e.g., to storie if in a cold place (2 to P) while avoiding freezing, to avoid shaking, and to remove particles with a first objection of the distributions of the proparation with the difficult to handle.

79 [9004] Accordingly, great concern has been directed toward the development of a pharmaceutical preparation having none of such limitations in handling.

[0005] The object of the invention is to provide a stable parenteral pharmaceutical composition or parenteral pharmaceutical composition or parenteral pharmaceutical programmation which comprises a humanization and propriess and the propriess and programmation which comprises a humanized monoclonal entitledy fragment and here no using immitations such as so old place preservation avoiding freezing, transfer and handling avoiding shaking, particle removing operation by a fifter when used and the like, and a method for the stabilization of the humanization monoclonal entitle or the stabilization of the stabilization of the promotion of the programmation of the programmation

Disclosure of the Invention

[0008] Under such a situation, the present inventors have conducted intensive studies and when ar fab fragment was obtained by papain digestion of a humanized C4G1 antibody produced by the method described in International Publication WO S013133 (corresponding U.S. Petant 577708), corresponding European Patent EP 619324) and then the Fab fragment was purified in accordance with the description in said specification, it was found that the three obtained humanized C4G1 Fab fragment can be stabilized by adding a nonionic surface active agent and secondands to the purified Fab fragment and adjusting pH of the mixture to a weakly addic level with a buffer, and the invention has been accomplished as a result of further confirmed studies. That is, the invention reletes to a parenteral pharmaceutical preparation which comprises a humanized monoclonal antibody fragment, a nonionic surface active agent and saccharides, wherein the pH is weakly acticit. Particularly, the invention relates to a parenteral pharmaceutical preparation which comprises a humanized monoclonal antibody fragment, as Fab fragment of a humanized monoclonal antibody for a fibrinogen receptor of a human platelet membrane glycoprotein C9IIIo/IIIa, a nonionic surface active agent and saccharides, wherein the pH is weakly acticit. The invention also relates to a method for the stabilization of the humanized monoclonal antibody fragment, which comprises formulating a nonronic surface active agent and addition file pH to a weakly acticit.

[0007] The humanized moredonal antibody fragment to be used in the invention is not particularly limited with the proviso that it generally has a therapeutically effective pharmacological action as a mediciament. For example, it may be any one of fragments prepared by making humanized monodonal entibodies described in JP-A-62-256800 (corresponding U.S. Pattent 5226509, corresponding European Pattent 239400, the term "JP-A" as used herein means an "unexamined published uponeen patent application"), International Publication WO 907661 (corresponding U.S. Patent 5593761, corresponding European Patent 451216) and the like into fragments by a method well known in sale technicals filled of g., a chemical technique, or enzymatic technique, and other molecular weight in or bardicularly limited, too. Also, it may be a fragment described, e.g., in WO 30/3133, which is directly produced by genetic engineering techniques. Preferred is a fragment having a platled aggregation inhibition action. As such a fragment, e.g., an Fab fragment of a humanized monodorial antibody for a fibrinogen receptor of a human platlett membrane glycoprotein GPILIFITIE are be cled. Illustratively, a humanized C4041 Fab fragment, prepared by digesting the humanized C404.

Fab entitlody produced by the method described in International Publication WO 93/13/133 with a protectyric enzyme (e.g., paperil) using a method well known in said technical field, thereby obtaining a reflat of well known in said technical field, thereby obtaining an Fab fregment, and the purplying the fragment in accordance with the description in said specification, is more desirable as such an Fab fragment (cf. the drawnes which will be described later).

5 [0008] According to the invention, the amount of the Fab fragment is not particularly limited, with the proviso that it is an amount capable of generally exerting a therapeutically effective pharmacological action as a medicament, but is preferably from 2 ms to 100 ms, more preferably from 6 ms to 50 ms.

[9009] According to the invention, the concentration of the Fab fragment is not particularly limited, with the provisor that it is generally within such a range that a parenteral pharmaceutical composition can be provided, but is prefurably from 0.01 mg/ml. to 10 mg/ml, more preferably from 0.1 to 8 mg/ml. When the concentration is lower than 0.01 mg/ml, there will be a case in which it is provision as a pharmaceutical preparation is difficult in reality, because the preparation becomes larger in size in order to keep the concentration for expressing effective pharmacological action. Also, when the concentration is higher than 10 mg/ml, it becomes close to the saturation solubility of the fragment, thus posing a cossibility of generating acceptance for the provision of the provision of the provision of the provision of the provision and provision of the provisi

[0010] The nonionic surface active agent to be used in the invention is not particularly limited with the proviso that it is generally phermaceutically acceptable. In this case, the nonionic surface active agent mehod does not show indice property, such as a polyalkylene glycol ether of an alightatic school, a polyalkylene glycol ether of an alikyl phenol or the like. For example, polysorbate 80, polysorbate 20 and the like can be cated, of which preferred is polysorbate 80 and more preferred is plant-originated polysorbate 80. The nonionic surface active agent of the invention can be formulated alone or as a combination of two or more species

[0011] According to the invention, the concentration of the nonionic surface active agent is not particularly limited, with the provisor that it is generally within such a range that a parenteral pharmaceutical composition can be provided, but is preferrably within a range of from about 1 x 10% by weight to 10 % by weight, more preferably from 0.001% by weight to 0.1% by weight, in the solution. When the concentration is lower than 1 x 10 % by weight, it will pose a possibility of generating aggregates by shaking. In this connection, the nonionic surface active agent of the invention is added manify to highly tornation of accreases the

[0012] The saccharides to be used in the invention are not particularly limited with the provise that they are generally pharmaceutically acceptable. Examples of such saccharides include glucose, xylose, galactose, fructose and the like monosaccharides, lactose, malose, purified sucrose, sucrose and the like disaccharides and mannifol, sorbitol, xylitid and the like sugar alcohols. Preferred are purified sucrose anxion mannifol. The saccharides of the invention can be formulated alone or as a combination of two or more. In this connection, the saccharides of the invention can be dominated to the stabilize the humanized monocional antibody and here functions, e.g., to adjust cannot pressure of the pharmaceutical preparation and to keep matrix components amorphous so that ra-dissolution of the preparation becomes seasy when it is freeze-diried.

100131 According to the invention, the concentration of the saccharides is not particularly limited, with the proviso that it is generally within such a range that a parenteral pharmaceutical composition can be provided, but is preferably from 0.01% by weight to 50% by weight, more preferably from 0.1% by weight to 10% by weight. When the concentration is lower than 0.1% by weight, it will pose a possibility of generating aggregates by shaking. Also, when it is higher than 50% by weight, there is a possibility that saccharides and the like are precipitated. In addition, when the concentration is within this range, the saccharides also exert the effect as a bulking agent when made into a freeze-dried preparation. [8014] According to the invention, pH of the parenteral pharmaceutical composition or preparation is not particularly limited when it is adjusted to a weakly acidic level by a known method. Preferably, the pH is adjusted to approximately from 4 to 6 by formulating a substance having the action to buffer at a weakly acidic level (to be referred to as a buffer hereinafter). According to the invention, the term weakly acidic means a pH value of approximately from 4 to 6. When the pH is from neutral to alkaline, stability of the preparation is considerably spoiled, such as increase in impurities. increase in aggregates and the like. Also, when the preparation is strongly acidic, it is desirable to avoid its use as mections due to pain and the like Examples of the buffer include a phosphate buffer (e.g., phosphoric acid-disodium hydrogenphosphate buffer), a citrate buffer (e.g., citric acid-sodium hydroxide), an acetate buffer (e.g., acetic acidsodium acetate), a tartarate buffer (e.g., tartario acid-sodium hydroxide), a malate buffer (e.g., malic acid-sodium hy-181 draxide), a histidine buffer (e.g., histidine-hydrochloric acid), an arginine buffer (e.g., arginine-hydrochloric acid) and the like. When the parenteral pharmaceutical composition is used as injections, their sodium salts are preferable, and sodium phosphate buffer and/or sodium citrate buffer is more preferable. These buffers of the invention may be used alone or as a combination of two or more.

[0015] According to the invention, the concentration of the buffer is not particularly limited, with the proviso that it is generally within such a range that a parenteral pharmaceutical composition can be provided, but is perferably 1 mM or more, preferably from 1 mM to 500 mM. When the concentration is lower than 1 mM, it is difficult to keep the pH stably due to too week buffer action. Since osmotic pressure becomes high when it exceeds 500 mM, the composition may be used by distingn with, e.g., distilled water for injection prior to use, but it is destrable that the concentration is

from 1 mM to 500 mM when the parenteral pharmaceutical composition or preparation is directly used without diluting prior to use.

[0016] The parenteral pharmacoulical preparation obtained by carrying out the invention is not particularly limited, with the proviso that it is in a generally pharmacoutically acceptable dosage form, but it is preferably in the form of assiptic preparations such as aqueous injections, nonequeous injections, injections to be dissolved prior to use (e.g., a preparation powdered by freeze drying method) and the like. In this connection, as the freeze-drying conditions, known conditions can be set outbonally

[0017] The parenteral pharmaceutical preparation of the invention is preserved generally in sealed containers as assplic preparations such as acqueous impetions, nonequeues injections, impetions to be dissolved grint to use (e.g., a preparation powdered by freeze drying method) and the like, and it is desirable that the space is under an oxygen-reduced atmospherer. In this case, the term "under an oxygen-reduced atmospherer is an atmosphere in which oxygen in the sit is artificially reduced. For this, it is desirable that the atmosphere in the sealed container is replaced, e.g., with an inert gas (e.g., nitrogen gas). More preferred is nitrogen gas. Also, the gas replacing ratio is preferably 90% or more, more preferably 90% or more.

(D18) Regarding the production method of the parenteral pharmaceutical preparation of the invention, a conventionally known method can be employed, and its example includes a method in which the Fab fragment produced by the method sessible of international Publication NV 09/31/333 is mixed with and dissolved in solution containing additives such as a nonionic surface active agent, secharides and the like, and the resulting solution is adjusted by mixing with a dilution buffer solution adjusted to result in the final concentration. In this case, since the final composition of the Fab fragment solution is influenced by the method of the final step of purification, the final concentration of each component can be optionally set by liquid composition-exchanging or concentrating the Fab fragment solution by diefficient on the like method.

[0019] The parenteral pharmaceutical composition of the invention can be mixed with pharmaceutical additives generally added to parenteral pharmaceutical compositions (e.g., a solubilizing agent, a preservative, a stabilizing agent and a tribic-aning agent, a soloring agent and a tribic-aning agent, a soloring agent and a tribic-aning agent). For example, cyclodexirins and the like can be cited as the solubilizing agent. Methyl p-berozate and the like can be cited as the re-undistlying agent. Energy alcohol and the like can be cited as the presentative. Lectifin and the like can be cited as the two local part of the like can be cited as the solution and the like and the like can be cited as the two cited as the solution append to like and the like can be cited as the two cited as the solution append to the like can be cited as the two cited as the solution append to the like can be cited as the two cited as the solution append to the like can be cited as the two cited as the solution append to the like can be cited as the two cited as the solution append to the like can be cited as the two cited as the solution append to the control of the like can be cited as the two cited as the solution append to the like can be cited as the two cited as the solution append to the like can be cited as the two cited as the solution appendix the control of the can be cited as the solution appendix the control of the control

Brief Description of the Drawings

[0020]

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Fig. 1 briefly illustrates the production process of a fragment (10) of a humanized monoclonal antibody.
Fig. 2 illustrates the action mechanism of a platelet aggregation inhibiting drug (platelet aggregation inhibitor).

[0021] As shown in Figs. 1 and 2, the humanized monoclonal antibody fragment (10) to be used in the invention is

produced by a process in which a monoclonal antibody for a fibringing neceptor (14) of a glycoprotein GPIIbillia existing on the surface of human platelet (12) is humanized (16) and then made into an Fab fragment by papain treatment (18)

(Description of the Reference Numbers and Signs)

[0022] 10: Fragment, 12: human platetet, 14: fibrinogen receptor, 16: humanized antibody, 18: papain freatment

Best Mode for Carrying Out the Invention

[0023] The following describes the invention further illustratively with reference to examples which, however, do not limit the scope of the invention.

[Reference Example]

[0024] An Fab fragment obtained by the following method was used as the humanized monocloral amilbody fragment.

That is, a humanized CAG1 antibody obtained by the method described in International Publication WO 93/13133

(Example) was digested by a papitin freatment to prepare an Fab fragment, and then the Fab fragment was purified in accordance with the description in said specification, thereby obtaining a humanized CAG1 Fab fragment (to be referred sample) to as "Fab fragment" (to be referred sample) to as "Fab fragment" (to be referred sample) to as "Fab fragment" (to the referred sample) to as "Fab fragmen

[Test Method 1] Titer measurement by binding-inhibition activity

[0025] Titers relative to a standard Fab fragment preparation are measured by atlowing a biolinylated fibrinogen solution and an Fab fragment solution to react competitively with a GPIIb/fila-immobilized plate and developing a color with an avoid neoroidase solution.

iTest Method 2] Determination of high molecular impurities by a high performance liquid chromatography

[0026] A 20 µl portion of a solution containing 1 mg of the Fab fragment is tested by a liquid chromatography under the following conditions. Peak areas are measured by an automatic integration method to calculate area percentages of peak areas other than that of the Fab fragment.

Detector: Ultraviolet absorption photometer

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Column; Dextran-covalent bonded agarose of 13 µm in particle size is packed in a glass tube having an inner diameter of about 10 mm and a length of about 30 cm.

Column temperature: Constant temperature at around 25°C

Mobile phase: A 3.12 g portion of sodium dihydrogenphosphate and 11.7 g of sodium chloride are dissolved in 900 ml of water, and the solution is adjusted to pH 7.0 by adding 8 N sodium hydroxide solution and then filled up to 1,000 ml.

20 Liquid quantity: Each sample is adjusted to such an amount that retention time of the Fab fragment peak becomes about 38 minutes.

[Test Method 3] Verification of appearance and aggregates by visual observation

25 [0027] The appearance of and the amounts of aggregates in liquid samples are compared by visual observation under an illumination intensity of from 2,000 lux to 5,000 lux

[inventive Examples 1 to 3] [Comparative Examples 1 to 5]

[0028] A purified preparation of the Fab fragment having a concentration of about 1 mg/ml was subjected to defiltration to change it to an Fab fragment aqueous solution having a concentration of from 3 to 6 mg/ml to be used as an Fab fragment bulk drug. Also, separately, various buffer solutions shown in Table 1 were prepared using respective components in such amounts that their concentrations at the time of final fill up became 2 mg/ml as the Fab fragment concentration, 10 mM as the buffer concentration, 0.01% by weight as the polysorbare 80 concentration and 9% by weight as the purified sucrose concentration, respectively, and make with the above bulk drug, thereby obtaining formulated sciutions. Each of these formulated solutions was subjected to seeptic filtration and then dispensed in 3 to 5 ml portions into previously sterilized vials under aseptic environment, the head space in each vial was replaced with introgen by repeating suction and de-suction in a lyophilization chamber, and then each of the resulting vials was sealed with a stopper to obtain pharmaceutical preparations of the invention. The inventive preparations and comparative preparations were storded at 40°C and 80°C to compare their stability.

[0029] The test results are shown in Table 1. As is evident from Table 1. high molecular weight impurities were increased and the samples became opaque under les ewere (night temperature) condition for the pit values of Comparative Examples, while reduction of liters was not observed by the pit values of the invention. Accordingly, it can be said that the observations of the revention are operations haven arreaded when stability.

(Table 1)

	(takes 1)						
	Buffer agent	pН	Results Upper column: titer (%) Middle column: property Lower column: high molecular impurit				
			Initial stage	40°C 1 month	60°C 4 weeks		
Inventive Ex. 1	Sodium phosphate	4.90	100	100	72		
			coloriess	*	coioriess		
			0.02	0.07	1.51		

(Table 1) (continued)

	Buffer agent	pH	Results Upper column: titer (%) Middle column: property Lower column: high molecular impurities (
			initial stage	40°C 1 month	60°C 4 weeks
Inventive Ex. 2	Sodium phosphate	5.95	100	100	81
			coloriess	-	coloriess
			0.03	0.10	1.08
Inventive Ex. 3	Sodium citrate	5.21	100	81	77
			coloriess		coloriess
			0.06	0.04	2.40
Comparative Ex. 1	Sodium phosphate	7.03	100	91	59
			coloriess	-	opaque
			0.03	0.17	1.69
Comparative Ex. 2	Sodium phosphate	7.85	100	-	*
			coloriess	*	opaque
			0.04	0.39	10.72
Comparative Ex. 3	Sodium citrate	7 14	100	68	56
			colorless		opaque
			0.13	0.13	2.78
Comparative Ex. 4	Sodium phosphate	9.04	100		-
			colorless	-	opaque
			0.04	1.48	62.06
Comparative Ex 5	Tris-HCI	7.16	100	83	80
			colorless	-	opaque
			0.07	1.62	0.64

[Inventive Examples 4 to 9] [Comparative Examples 6 and 7 (polysorbate 80)]

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[033]. A purified preparation of the Fab fragment having a concentration of about 1 mg/ml was subjected to diaffitration to change it to an Fab fragment aqueous solution having a concentration of from 3 to 6 mg/ml to be used as an Fab fragment buck drug. Also, separately, a buffer solution was prepared using respective components in such amounts that their concentrations at the time of first final flut became? Engined is the Feb fragment concentration 10 mM as the soldium prosphate concentration and 5% by weight as the purified sucrose concentration; respectively, and mixed with the above butk drug, thereby obtaining formulated solutions. Each of these formulated solutions was subjected to septic filtration and them dispensed in 3 to 5 ml protions into previously settilized value under seeptic environment, the head space in each value was replaced with nitrogen by repeating suction and de-suction in a lyophilization chamber, and then each of the resulting value was seeded with a stopper to obtain pharmaceutical preparations of the invention. The inventive preparations and comparative preparations were shaken at 200 rpm for 10 minutes to verify the presence or abscince of sucreastie formulations.

[9031] The test results are shown in Table 2. As is evident from Table 2, aggregate formations can be considerably reduced by the adatton of 1×10^{-56} by weight or more of polysorbate 80 even when shaking or the like physical stress was applied.

(Table 2)

		(lable 2)	
	ionic surface ac	tive agent	Visual confirmation of aggregates
	Kind	Concentration (% by weight)	
Inventive Ex. 4	polysorbate 80	1	ne
Inventive Ex. 5	polysorbate 80	0.1	no
Inventive Ex. 6	polysorbate 80	0.01	no
Inventive Ex. 7	polysorbate 80	0 001	no
Inventive Ex. 8	polysorbate 80	0.0001	no
Inventive Ex. 9	polysorbate 80	0.00001	no
Comparative Ex. 6	polysorbate 80	0.000001	yes
Comparative Ex. 7	polysorbate 80	0	yes

(inventive Example 10) (Comparative Example 8 (saccharides))

[9032] A purified preparation of the Fab fragment having a concentration of about 1 mg/ml was subjected to disfilitration to brienge it to an Fab fragment aqueous exhition having a concentration of from 3 to 6 mg/ml to be used as an Fab fragment bulk drug. Also, experately, a buffer solution was prepared using respective components in such amounts that their concentrations at the time of first fill up became 2 mg/ml as the Fab fragment concentration. The wild as the soldium prosphete concentration and 0.01% by weight as the polysorbate 80 concentration, respectively, and mixed with the above bulk drug, thereby obtaining formulated solutions. Each of these preparations solutions was surjected to aseptic filtration and then dispensed in 3 to 5 ml portions into previously sterificate valual under assignt environment, the head space in each val was replaced with introgen by repeating suction and de-suction in a lyophifization chamber, and then each of the resulting visic was sealed with a stopper to obtain pharmaceutical preparations of fine invention. The inventive preparation and comparative preparation were stored at 60°C for 4 weeks to verify the presence or absence of aggregate formations.

[9033] The test results are shown in Table 3. As is evident from Table 3, it was able to prevent generation of aggregates during preservation by the inventive pharmaceutical preparation of the invention to which purified sucross was added

(Table 3)

		Saccharide		Visual confirmation of aggregates
		Kind	Concentration (% by weight)	
inveni	live Ex. 10	purified sucrose	5	no
Comp	arative Ex. 8	absent	0	insoluble foreign matter

(inventive Example 11)

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[0034] A purified preparation of the Fab fragment having a concentration of about 1 mg/ml was subjected to diaffitration to change if to an Fab fragment auteous solution having a concentration of from 31 50 mg/ml to be used as an Fab fragment bulk drug. Also, separately, a buffer solution was prepared using respective components in such amounts that their concentrations at the time of final fill up became 2 mg/ml as the Fab fragment concentration, 10 mlA as the sodium phosphate concentration, 0.01% by weight as the polysothet 80 concentration and 5% by weight as the purified sucrose concentration, respectively, and mixed with the above bulk drug, thereby obtaining a preparation solution. This preparation solution and then dispensed in 3 to 5 ml politions into previously sterilized vials under asseptic environment, the head space in each val was replaced with nitrogen by 95% by repeating suction and de-auction in a typohilization chamber, and then each of the resulting vials was sealed with a slopper to obtain a pharmaceutical preparation. This was stored at 40°C and 60°C to compare the stability during respensions. European Patent Office Office européen des brevets



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(54) PARENTERAL MEDICINAL COMPOSITION CONTAINING HUMANIZED MONOCLONAL ANTIBODY FRAGMENT AND METHOD FOR STABILIZING THE SAME

(57) This invention relates to a parenteral pharmaceutical composition which comprises a humanized monoclonal antibody fregment, a nonlonic surface active agent and saccharides, wherein its pft is weatly actide. The invention also relates to a method for the stabilization of a humanized antibody fragment, which comprises formulating a nonlonic surface active agent and seachandes and adulating the pit to a weakly sodie level

According to the invention, a stable parenteral pharmaceutical composition or parenteral pharmaceutical composition or parenteral pharmaceutical properties of the pharmaceutical monodonal antibody fragment and has no using ilinitations such as cold place preservation avoiding freezing, transfer and handling avoiding shaking, particle removing operation by a filter when used and the like.

Description

fechnical Field

[0801] This invention relates to a stable parenteral pharmaceutical composition which comprises a humanized monocional antibody fragment. Particularly, the invention relates to a stable parenteral pharmaceutical composition which comprises Rab fragment of a humanized monocional antibody for a fibringoen receptor of a human platefet membrane glycoprotein GPilibilia. The invention also relates to a method for the stabilization of a humanized monocional antibody fragment, which comprises formulating a nonionic surface active agent and saccharides and adjusting the pH to a weakly accide level.

Background Art

[6002] Since the proposal of a method for the mass production of monoclonal antibodies by means of genetic engineering, monocloral antibodies have been broadly used in the field of medicaments.

[9033] "Recently, Centocor in the United States has developed "ReoPro, (trade name)" comprised of a human-mouse chimeric monoclonal antibody fragment and is providing it as a platelet aggregation inhibitor in the clinical field This pharmacoulted preparation is a liquid preparation of pH 7.2 containing 2 mg/ml of a chimeric monoclonal antibody fragment, 0.01 M of sodium phosphate, 0.15 M of sodium chiotide and 0.001% of polysorbate 80. Since this preparation is a liquid preparation, it is not necessary to use it by dissolving in distilled water for rijection or the tike prior to use as in the case of freeze-dired preparations. However, this preparation has some limitations in handling it, e.g., to store it in a cold place (20 th) while soviding freezing, to avoid shaking, and to remove particles with a fifter prior to its administration. Because of such limitations, it is considered that this preparation is a preparation which is difficult to handle

25 [0004] Accordingly, great concern has been directed toward the development of a pharmaceutical preparation having note of such limitations in handling.

[0005] The object of the invention is to provide a stable parenteral pharmaceutical composition or parenteral pharmaceutical composition or parenteral pharmaceutical preparation which comprises a humanized monoclonial antibody fragment and has no using limitations such as so oid place preservation avoiding freezing, transfer and handling avoiding shaking, particle removing operation by a fatur when used and the tike, and a method for the stabilization of the humanized monoclonial antibody fragment

Disclosure of the Invention

(8008) Under such a situation, the present inventors have conducted intensive studies and when an Fab fragment was obtained by papain digestion of a humanized C4G1 antibody produced by the method described in international Publication WO 89/13/133 (corresponding U.S. Patent 57/7085, corresponding European Patent EF 6/13024) and then the Fab fragment was putified in accordance with the description in said specification, it was found that the thrus obtained humanized C4G1 Fab fragment can be stabilized by adding a nonionic surface active agent and saccharides to the purified Fab fragment and adjusting pH of the mixture to a weakly actific level with a buffer, and the invention has been accomplished as a result of further continued studies. That is, the invention relates to a parenteral pharmaceutical proparation which comprises a humanized monoclonal antibody fragment, a nonionic surface active spert and saccharides, wherein the pH is weakly actici. Particularly, the invention relates to a parenteral pharmaceutical proparation which comprises a humanized monoclonal antibody fragment, as Fab fragment of a humanized monoclonal antibody for a fibrinogen receptor of a human platelet membrane glycoprotein OPIIbiflia, a nonionic surface active agent and saccharides, wherein the pH is weakly actici. The invention also relates to a method for the stabilization of the humanized monoclonal antibody fragment, and solve the stabilization of the humanized monoclonal antibody fragment, which comprises formulating a nonionic surface active agent and saccharides and adjusting the pH to a weakly acticitie very.

19007] The humanized monodional antibody fragment to be used in the invention is not particularly limited with the provise that it penerally has a therapeutically feferive pharmacological action as a mediciament. For example, it may be any one of fragments prepared by making humanized monoclonal antibodies described in JP-A-62-295990 (corresponding U.S. Paterns 5225539, corresponding European Patent 293040), the term *JP-A* as used herein means an "inversamined published Japanese patent application"), international Publication WO 907861 (corresponding U.S. Patent 5525761, corresponding European Patent 451216) and the ilie into fragments by a method well known in said technical feel (e.g., a chemical technique) and their indecative weight is not particularly limited, too. Also, it may be a fragment described, e.g., in WO 90713133, which is directly produced by geneite engineering echniques. Preferred is a fragment having a platelet aggregation minibilities notion. As such a fragment, e.g., an Fab fragment of a humanized dood.

Fab antibody produced by the method described in International Publication WO 93/13133 with a proteolytic enzyme (e.g., papain) using a method well known in said technical field, thereby obtaining an Fab fragment, and then purifying the fragment in accordance with the description in said specification, is more desirable as such an Fab fragment (of the drawings which will be described later).

- [0008] According to the invention, the amount of the Fab fragment is not particularly limited, with the proviso that if is an emount capable of generally exerting a therapeutically effective pharmacological action as a medicament, but is preferably from 2 mg to 100 mg, more preferably from 5 mg to 50 mg.
 - [0009] According to the invention, the concentration of the Fab fragment is not particularly limited, with the provision that it is generally within such a range that a parenteral pharmacoutical composition can be provided, but is picetably from 0.01 mg/ml; but 10 mg/ml, more preferably from 0.1 to 8 mg/ml. When the concentration is lower than 0.01 mg/ml, there will be a case in which its provision as a pharmaceutical preparation is difficult in reality, because the preparation becomes large in size in order to keep the concentration for expressing effective pharmacological action. Also, when the concentration is higher than 10 mg/ml, it becomes close to the saturation solubility of the fragment, thus posing a possibility of generating aggregates during preservation.
- 15 [0014] The noninnic surface active agent to be used in the invention is not particularly limited with the proviso that it is generally phermaceutically acceptable. In this case, the nonionic surface active agent means a surface active agent which does not show ionic property, such as a polyalkylene glycol either of an aliphatic alcohol, a polyalkylene glycol either of an alkyl pheniol or the like. For example, polyacohote 60, polysorbate 22 and the like can be titled, of which preferred is polyacohate 80 and more preferred is plant-originated polyacohote 80. The nonionic surface active
 - agent of the invention can be formulated alone or as a combination of two or more species.

 [0011] According to the invention, the concentration of the nonincie surface active agent is not particularly limited, with the provise that it is generally within such a range that a parenteral pharmaceutical composition can be provided, but is preferably within a range of from about 1 x 10% by weight, in the preferably within a range of from about 1 x 10% by weight, in x 10% by weight, in the solution. When the concentration is lower than 1 x 10% by weight, it will pose a possibility of generating aggregates by shaking, in this connection, the nonionic surface active agent of the invention is added mainty to shiplit formation of segregates.
 - Object The saccharides to be used in the invention are not particularly limited with the proviso that they are generally pharmaceutically acceptable. Examples of such saccharides include glucose, xylose, galactose, fluctose and the like knooseacharides, lactose, malicose, purified surrose, sucrose and the like disuscentides and mannitol, surbitol, xylitol and the like sugar alcohols. Preferred are purified sucrose and/or mannitol. The saccharides of the invention can be formulated alone or as a combination of two or more. In this connection, the saccharides of the invention are added mainly to stabilize the humanized monoclonal antibody and have functions, e.g., to adjust osmotic pressure of the pharmaceutical preparation and to keep matrix components amorphous so that re-dissolution of the preparation becomes assay when it is freeze-dried.
- 36 [8013] According to the invention, the concentration of the secchandes is not particularly limited, with the proviso that it is generally within such a range that a parenteral pharmaceutical composition can be provided, but is preferably from 0.01% to yeepith to 50% by weight, the one preferably from 0.1% by tweight to 10% by weight of 10% by weight of 10% by weight of 10% by weight of 10% by weight, it will pose a possibility of generating aggregates by shaking. Also, when it is higher than 50% by weight, there is a possibility that sacchardes and the like are precipitated. In addition, when the concentration is within this range, the secondaries also exert the effect as a bulking agent when made into a freeze-dried preparation.
- 10044] According to the invention, pit of the parenteral pharmaceutical composition or preparation is not particularly imitted when it is adjusted to a weakly acticl level by a known method. Proferably, the pit is adjusted to a proximately from 4 to 6 by formulating a substance having the action to buffer at a weakly acticl level (to be referred to as a buffer the pit is form a to 6 by formulating a substance having the action to buffer at a weakly acticl level (to be referred to as a buffer the pit is form neutral to alialized, stability of the preparation is considerably spoiled, such as increase in impurities, increase in aggregates and the like. Also, when the preparation is strongly acticle, it is desirable to evoid its use as injections cut so to pain and the like. Examples of the buffer include a phosphate buffer (e.g., phosphora actic-disordium hydrogenphosphate buffer (e.g., that buffer (e.g., decir actic-action many hydroxide), an actate buffer (e.g., mails actid-action hydroxide), a historia buffer (e.g., historia buffer (e.g., historia composition is used as injections, there social many actions are perfectable, and the like When the parenter alpharmaceutical composition is used as injections, their social same preferable, and
- alone of as a combination of two or more. [8015] According to the invention, the concentration of the buffer is not particularly limited, with the proviso that it is generally within such a range that a parenteral pharmaceutical composition can be provided, but is preferably 1 mM or more, preferably from 1 mM to 500 mM. When the concentration is lower than 1 mM, it is difficult to keep the pH statily due to too wash buffer action. Since asmotic pressure becomes high when it exceeds 500 mM, the composition may be used by difficulty with, e.g., distilled water for injection prior to use, but it is desirable that the concentration is

sodium phosphate buffer and/or sodium citrate buffer is more preferable. These buffers of the invention may be used

from 1 mM to 500 mM when the parenteral pharmaceutical composition or preparation is directly used without diluting

[0015] The parentersi pharmaceutical preparation obtained by carrying out the invention is not particularly imitted, with the proviso that it is in a generally pharmaceutically acceptable desage form, but it is preferably in the form of asopiic preparations such as aqueous injections, nonequeous injections, injections to be dissolved prior to use (e.g., a preparation powdered by freeze drying method) and the like. In this connection, as the freeze-drying conditions, known conditions can be set obtomally.

[0017] The parenteral pharmaceutical preparation of the invention is preserved generally in sealed containers as a septic preparations auch as aqueous injections, nonaqueous injections, injections to be dissolved prior to use (e.g., a preparation provided by freeze drying method) and the like, and it is desirable that the space is under an oxygenreduced atmosphere. In this case, the term "under an oxygen-reduced atmosphere" means an atmosphere in which oxygen in the sit is artificially reduced for this, it is desirable that the atmosphere in the sealed container is replaced, e.g., with an inert gas (e.g., nitrogen gas). More preferred is nitrogen gas. Also, the gas replacing ratio is preferably 90% or more, more preferably 90% or mor

[0018] Regarding the production method of the parenteral pharmaceutical preparation of the invention, a conventionally known method can be employed, and as example includes a method in which the Fab fragment produced by the method described in international Publication WIO 93/13133 is mixed with and dissolved in solution confeiring additives such as a nonitione surface active agent, seccharides and the like, and the resulting solution is adjusted by mixing with a distallon buffer solution adjusted to result in the final connentration. In this case, since the final composition of the Fab fragment solution is influenced by the method of the final step of purification, the final concentration of each component can be optionally set by liquid composition-exchanging or concentrating the Fab fragment solution by distribution of the kills method.

[0319] The parenteral pharmaceutical composition of the invention can be mixed with pharmaceutical additives generally added to parenteral pharmaceutical compositions (e.g., a solubilizing agent, a preservative, a stabilizing agent, an emularity agent, a southing agent, at the parent agent, a buffing agent, a coloring agent and a thick-ening agent). For example, cyclodextrins and the like can be cited as the solubilizing agent. Methyl p-berzoutic and the like can be cited as the resultarity agent. Benzy alcohol and the like can be cited as the resultarity agent. Methyl agent. Sodium chloride and the like can be cited as the tonicity sgent. Maltice and the like can be cited as the present and the like can be cited as the two parents.

Brief Description of the Drawings

[0020]

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Fig. 1 briefly illustrates the production process of a fragment (10) of a humanized monoclonal antibody.

Fig. 2 illustrates the action mechanism of a platelet aggregation inhibiting drug (platelet aggregation inhibitor).

[0021] As shown in Figs. 1 and 2, the humanized monoclonal antibody fragment (10) to be used in the revention is produced by a process in which a monoclonal antibody for a fibringen receiptor (14) of a glycoprotein GPIIb/Illa existing on the surface of human plateful (21) is humanized (16) and then made into an Fab fragment by papain treatment (19).

(Description of the Reference Numbers and Signs)

[9022] 10: Fragment, 12. human platelet, 14: fibrinogen receptor, 16: humanized antibody, 18: papain treatment

Best Mode for Carrying Out the Invention

[0023] The following describes the invention further illustratively with reference to examples which, however, do not limit the scope of the invention.

[Reference Example]

[9024] An Fab fragment obtained by the following method was used as the humanized monocional antibody fragment. That is, a humanized C4G1 antibody obtained by the method described in International Publication (Vio 29131313 (Example) was digested by a papain treatment to prepare an Fab fragment, and then the Fab fragment was purified in accordance with the description in said specification, thereby obtaining a humanized C4G1 Fab fragment (to be referred simply to as Fab fragment Theorienfert).

(Test Method 1) Titer measurement by binding-inhibition activity

[0025] Titers relative to a standard Fab fragment preparation are measured by allowing a biotinylated fibrinogen solution and an Fab fragment solution to react competitively with a GPIIb/IIIa-immobilized plate and developing a color with an avoid neoroxidase solution.

[Test Method 2] Determination of high molecular impurities by a high performance liquid chromatography

[9026] A 20 µl portion of a solution containing 1 mg of the Fab fragment is tested by a liquist chromatography under the following conditions. Peak areas are measured by an automatic integration method to calculate area percentages of peak areas other than that of the Fab fragment.

Detector, Ultraviolet absorption photometer

- Column: Dextran-covalent bonded agarose of 13 µm in particle size is packed in a glass tube having an inner diameter of about 10 mm and a length of about 30 cm.
- Column temperature: Constant temperature at around 25°C
 - Mobile phase: A 3.12 g portion of sodium dihydrogenphosphate and 11.7 g of sodium chloride are dissolved in 900 ml of water, and the solution is adjusted to pH 7.0 by adding 8 N sodium hydroxide solution and then filled up to 1 0.00 ml.
- 20 Liquid quantity; Each sample is adjusted to such an amount that retention time of the Fab fragment peak becomes about 38 minutes.

(Test Method 3) Verification of appearance and aggregates by visual observation

26 [0027] The appearance of and the amounts of aggregates in liquid samples are compared by visual observation under an illumination intensity of from 2,000 tux to 5,000 tux.

[inventive Examples 1 to 3] [Comparative Examples 1 to 5]

20 [0028] A purified preparation of the Fab fragment having a concentration of about 1 mg/ml was subjected to distilutation to change it to an Fab fragment apueue as solubin having a concentration of from 3 to 6 mg/ml to be used as an Fab fragment bulk drug. Also, separately, various buffer solutions shown in Table 1 were prepared using respective components in such amounts that their concentrations at the time of final fill up became 2 mg/ml as the Fab fragment concentration. 10 ml Ma site buffer concentration, 0.01% by weight as the polysorbate 80 concentration and 5% by weight as the pulified sucrose concentration, respectively, and mixed with the above bulk drug, thereby obtaining formulated solutions. Each of these formulated solutions was subjected to asseptic filtration and then dispensed in 3 to 5 ml portions into previously startized vals under asseptic environment, the head space in each vial was replaced with a stopper to obtain pharmaceutical preparations of the invention. The inventive preparations and comparative preparations were stored at 40°C and 60°C to compare their stability.

[0029] The test results are shown in Table 1. As is evident from Table 1, high molecular weight impurities were increased and the samples became opaque under the severe (high temperature) condition for the pri-values of Comparative Examples, while reduction of tiers was not observed by the pri-values of the invention. Accordingly, it can be said that the pharmaceutical preparations of the invention are preparations having markedly high stability.

(Table 1)

(1457-1)							
	Buffer agent	pН	Results Upper column: titer (%) Middle column: property Lower column: high molecular impurities (%)				
			Initial stage	40°C 1 month	60°C 4 weeks		
Inventive Ex. 1	Sodium phosphate	4.90	100	100	72		
			coloriess	-	coloriess		
			0.02	0.07	1.51		

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(Table 1) (continued)

	Buffer agent	pН	Results Upper column: titer (%) Middle column: property Lower column: high molecular impurities (*)		
			Initial stage	40°C 1 month	60°C 4 weeks
Inventive Ex. 2	Sodium phosphate	5.95	100	100	81
			coloriess	-	coloriess
			0.03	0.10	1.08
Inventive Ex. 3	Sodium citrate	5.21	100	81	77
			colorless	-	coloriess
			0.06	0.04	2.40
Comparative Ex. 1	Sodium phosphate	7.03	100	91	59
			colorless	-	opaque
			0 03	0.17	1.69
Comparative Ex. 2	Sodium phosphate	7.85	100	-	-
			coloriess	+	obsdne
			0.04	0.39	10.72
Comparative Ex. 3	Sodium citrate	7.14	100	68	56
			colorless	-	opaque
			0.13	0.13	2.78
Comparative Ex. 4	Sodium phosphate	9.04	100	-	-
			colorless	-	opaque
			0.04	1.48	62.06
Comparative Ex. 5	Tris-HCI	7.16	100	83	80
			colorless		opaque
			0.07	1.62	0.64

[Inventive Examples 4 to 9] [Comparative Examples 6 and 7 (polysorbate 80)]

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[0030] A purified preparation of the Fab fragment having a concentration of about 1 mg/ml was subjected to daffitration to change it to an Fab fragment aqueous solution having a concentration of from 3 to 6 mg/ml to be used as an Fab fragment and the subject of the subject

[0031] The lest results are shown in Table 2. As is evident from Table 2, aggregate formations can be considerably reduced by the addition of 1 x 10 % by weight or more of polysorbate 80 even when sheking or the like physical stress was applied.

Children 20

		(lable 2)	
	ionic surface act	tive agent	Visual confirmation of aggregates
	Kind	Concentration (% by weight)	
Inventive Ex. 4	polysorbate 80	1	по
Inventive Ex. 5	polysorbate 80	0.1	no
Inventive Ex. 6	polysorbate 80	0.01	no
Inventive Ex. 7	polysorbate 80	0.001	no
Inventive Ex. 8	polysorbate 80	0.0001	on
Inventive Ex 9	polysorbate 80	0.00001	no no
Comparative Ex. 6	polysorbate 80	0.000001	yes
Comparative Ex. 7	polysorbate 80	0	yes

[Inventive Example 10] [Comparative Example 8 (saccharides)]

[0032] A purified preparation of the Fab fragment having a concentration of about 1 my/mi was subjected to disflitration to change it to an Fab fragment alequeous solution having a concentration of from 3 to 6 my/mi to be used as an Fab fragment bulk drug. Also, separately, a buffer solution was prepared using respective components in such amounts that their concentrations at the time of final fit up became 2 my/mi as the fab fragment concentration. May as the soldium phosphate concentration and D01% by weight as the polysorbate 80 concentration, respectively, and mixed with the above bulk drug, thereby obtaining formulated solutions. Each of these preparation solutions was subjected to aseptic fiftients and then dispensed in 3 to 5 mill portions into previously sterificate vists under assigne environment, the need space in each vist was explaced with nitrogen by repeating suction and de-suction in a lyophilization chambur, and then each of the resulting vista was sealed with a stopper to obtain pharmaceutical preparations of the invention. The inventive preparation and comparative preparation were stored at 60°C for 4 weeks to verify the presence or sessence of gancerates formations.

(1003) The test results are shown in Table 3. As is evident from Table 3, it was able to prevent generation of aggregates during preservation by the inventive pharmaceutical preparation of the invention to which purified sucrose was added.

(Table 3)

	Saccharide		Visual confirmation of aggregates
	Kind Concentration (% by weight)		
Inventive Ex. 10	purified sucrose	5	no
Comparative Ex. 8	absent	0	insoluble foreign matter

Inventive Example 111

(0044) A purified preparation of the Fab fragment having a concentration of about 1 mg/ml was subjected to disflitration to change it to an Fab fragment equeous solution having a concentration of from 3 to 6 mg/ml to the used as an Fab fragment bulk drug. Also, separately, a buffer solution was prepared using respective comprovers in such amounts that their concentrations at the time of final fill up became 2 mg/ml as the Fab fragment concentration. Mid as the sodium phosphate concentration, 0.01% by weight as the polysotate 90 concentration and 9% by weight as the purified sucrose concentration, respectively, and mixed with the above bulk drug, thereby obtaining a preparation solution. This preparation solution was subjected to asseptic filtration and then dispensed in 3 to 5 ml portions into previously sternized vials under asseptic environment, the head space in each vial was replaced with introgen by 95% by repeting suction and de-suction in a typolification chamber, and then each of the resulting vials was seated with a stopper to obtain a pharmaceutical preparation. This was stored at 40°C and 50°C to compare the stability during preservation.

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position of any one of claims 1 to 11.

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- A parenteral pharmaceutical preparation in which the parenteral pharmaceutical composition of any one of claims to 12 is preserved in an oxygen-reduced atmosphere.
- 14. A method for stabilizing a humanized monoclonal antibody fragment, which comprises formulating a nonionic surface active agent and saccharides and adjusting the pH to a weakly acidic level.
- 15. The method for stabilizing a humanized monoclonal antibody fragment according to claim 14, wherein it is further preserved in an oxygen-reduced atmosphere.

FIG. 1

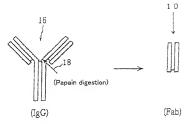
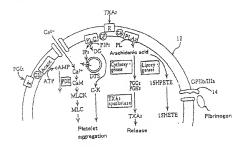


FIG. 2



PL: phospholipid, PLC: phospholipase C, PLA2: phospholipase A2,

G: GTP binding protein, GD: 1,2-diacylglycerol,

IP3: inositol 1,4,5-triphosphate, CaM: calmodulin,

MLCK: myosin light chain kinase, MLC: myosin light chain,

C-K: protein C kinase, FDE: phosphodiesterase,

DTS: dense tubular system

INTERNATIONAL SEARCH REPORT

International application No.

			PCT/J	P00/02784				
	A. CLASSIRCATION OF SUBHICT MATTER Inu.Cl ⁺ ASIXS9/395, 9/09, 9/19, 47/26, 47/34//COTX15/36 Cl2P21/08							
	o international Pasent Classification (tPC) or to both na	tional classification as	id IPC					
	S SEARCHED							
Monimum d Int.	ocumentation searched (classification bystem followed .Cl. A61K39/395, 9/08, 9/19, 47 Cl2P21/08							
Documental	tion searched other than minimum documentation to the	extent that such does	ments are included	in the fields searched				
CAPI	Rus base consulted during the international search (num LUSA (STN) , MEDLINE (STM) , EMBASE (STI ST (JOIS) , WPI-L(QUESTEL)	e of data base and, wh N), BIOSIS (STN	ere precioable, sca () ,	rch terms used)				
C. DOCU	MENTS CONSIDERED TO BE RELEVANT							
Cutegory*	Citation of document, with indication, where ap		nnt passages	Relevant to claim No.				
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Date of the 28	across completion of the international search July, 2008 (28.07.90)	Daw of mailing of the Daw S R August	e international sear L, 2000 (08					
Name and	mailing address of the ISAV	Authorized officer						

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INTERNATIONAL SEARCH REPORT

international application No.
PCT/JP00/02784

		1200/02/64
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lategory*	Charlon of document, with indication, where appropriate, of the relevant passages	Relevant to claim N
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(Lable 3)					
			Results (%)	Upper colu	mn: titer
-	Nitrogen replaceme	pН	mala	Lower colu	
1	. ~	l		impurities	(%)
	nt	ĺ	Initial	40°C	40°C
			stage	3 months	6 months
Inventive Ex.	yes	5.95	100	92	85
11			0.02	0.29	0.67

Industrial Applicability

190351. The parenteral pharmaceutical composition or preparation of the invention exerts excellent effects under a liquid state or a freeze-dried state, namely, it shows excellent preservation stability, it can be stored at room temperature, it inhibits aggregate formations so that it does not require particle removing step by a filter, and it can be used conveniently

Claims

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- A parenteral pharmaceutical composition which comprises a humanized monoclonal antibody fragment, a nonionic 25 surface active agent and saccharides, wherein the pH is weakly acidic.
 - 2. The parenteral pharmaceutical composition according to claim 1, wherein the humanized monoclonal antibody fragment has an action to inhibit aggregation of human platelets.
- 30 3. The parenteral pharmaceutical composition according to claim 1 or 2, wherein the humanized monoclonal antibody fragment is an Fab fragment of a humanized monoclonal antibody for a fibrinogen receptor of a human platelet membrane glycoprotein GPIIb/IIIa.
- 4. The parenteral pharmaceutical composition according to claim 3, wherein the concentration of the Fab fragment 35 is from 0.01 mg/ml to 10 mg/ml.
 - 5. The parenters) pharmaceutical composition according to any one of claims 1 to 4, wherein it is further blended with a substance having an action to buffer weakly acidic to adjust the pH value approximately 4 to 6.
- 8. The parenteral phermaceutical composition according to claim 5, wherein the substance having an action to buffer weakly acidic is sodium phosphate and/or sodium citrate.
 - 7. The parenteral pharmaceutical composition according to any one of claims 1 to 6, wherein the concentration of the substance having an action to buffer weakly acidic is from 1 mM to 500 mM
 - 8. The parenteral pharmaceutical composition according to any one of claims 1 to 7, wherein the nonionic surface active agent is polysorbate 80.
- 9. The parenteral pharmaceutical composition according to any one of claims 1 to 8, wherein the concentration of 30 the nonionic surface active agent is from 1 x 10-5% by weight to 1% by weight.
 - 19. The parenteral pharmaceutical composition according to any one of claims 1 to 9, wherein the seccharides are purified sucrose and/or mannitol.
 - 11. The parenteral pharmaceutical composition according to any one of claims 1 to 10, wherein the concentration of the saccharides is from 0.01% by weight to 50% by weight
 - 12. A parenteral pharmaceutical preparation which is produced by freeze-drying the parenteral pharmaceutical com-